Trans-epithelial cross-linking with riboflavin solution: two-year clinical results

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Material reported in this article has been presented at the 2nd Joint International Congress Refractive Online & SICSSO, Rome 2012 (podium presentation).

Purpose: to report the clinical results up to 24 months after Trans-Epithelial Cross-Linking (TE-CXL) performed on keratoconus-affected eyes, using a new riboflavin solution with enhancers of the epithelial permeability, and a new treatment protocol.

Study Design

- Clinical, perspective, non-randomized study;
- Conducted according to the ethical standards of the Declaration of Helsinki (revised in the year 2000). The patients were informed about the nature and the aim of experimentation, and subscribed an informed consent;
- 19 patients (11 male, 8 female) for a total of 25 eyes (13 right, 12 left);
- Mean age 26.68 +/- 7.44 years;
- All eyes affected by keratoconus, progressive in the last 6 months.

Exclusion criteria:

- age <16 or >45 years;
- Evidence of clinical and instrumental stability of keratoconus in the last 6 months;
- Thinnest corneal point <350 microns;
- Corrected visual acuity <20/40;
- Evidence of sub-epithelial or mid-anterior stromal scars or opacities;
- Evidence of marked Vogt striae or confocal evidence of deep reticular pattern dark bands;
- Associated corneal diseases; - previous ocular surgery procedures;
- Wearing of contact lenses in the last four weeks;
- Pregnant or breast feeding women;
- Poor compliance.

Clinical observations were performed one week before TE-CXL and at one, three, six, twelve and 24 months after treatment. Every observation included:

- Uncorrected (UCVA) and best spectacle corrected (BSCVA) visual acuity, measured in mesopic condition with LogMar chart;
- Spherical (SPH), cylindrical (CYL) and sphero-equivalent (SE) correction;
- Topographic measurement of corneal curvature parameters: simulated keratometry (SIMK), maximum (KMAX), minimum (KMIN) and mean (KM) corneal curvature, (Tomey TMS 4, Tomey Corporation, Japan; Orbscan IIz, Bausch & Lomb, USA);

- Clinical (slit-lamp) examination of anterior and posterior segment;
- Measurement of intra-ocular pressure (IOP) with Goldmann applanation tonometer;
- Endothelial corneal cell count (Tomey EM-3000, Tomey Corporation, Japan);
- Ultrasound measure of thecorneal central thickness (CCT), (Quantel Medical, Clermont-Ferrand, France);
- Confocal microscopy (CS4 Confoscan, Nidek Technology, Japan);
- Ocular coherence tomography of the cornea (Visante, Zeiss, Germany).

Pre-treatment therapy and protocol

Three days before treatment:

- preservative-free norfloxacin 0.3% eye drop, one drop every 6 hours.

Starting twenty minutes before treatment:

- Local anesthesia (oxybuprocaine hydrochloride 0,2% two drop every 5 minutes);
- Antibiotic prophylaxis (one drop of norfloxacin 0.3% every three minutes);
- Protection of posterior ocular structures with miosis (two eyedrops of 1% pilocarpin);
- Disinfection of periocular skin with iodine povidone 10% solution.

Treatment protocol

- Silicone ring (12 mm. diameter, 3 mm. height) placed on the corneo- scleral limbus;
- Riboflavin solution (riboflavin-dextran 0.1 g/100 g, D-alpha-tocopheryl polyethylene-glycol 1000 succinate (vitamin E TPGS) 500 mg/100 ml, coenzyme Q 100 mg/100 ml);
- The ring filled with the riboflavin solution was maintained for 15 minutes; further drops added if necessary to maintain the soaking of corneal tissue. - UV-A radiation was then applied after the positioning of a speculum, without any epithelium removal;
- VEGA CBM X-Linker® (CSO, Italy) as UV-A emitter (370 nm. wavelength); - the energy was lowered with partially absorbing UV-filters, to achieve 1.5 mW/cm2, measured with UV-meter (Peak Tech 5085);
- Treatment diameter: 8 mm, at 5 cm. distance from corneal apex;
- Two irradiation steps of 5 minutes were performed.
- No further riboflavin solution applied during UV-A exposure;
- Washing of the corneal surface with balanced salt solution (BSS) to remove the superficial riboflavin film before irradiation;
- Delivering of BSS drops on the corneal surface during UV exposure to maintain an adequate moisture.
- Norfloxacin 0,3% eyedrops administered at the end of the treatment.

Post-treatment therapy

- Topical antibiotic and lubricant therapy (norfloxacin 0.3% and hyaluronate 0.15% eye drops every six hours).

Results

- No severe side effects were observed during follow-up;
- One case of corneal haze and two cases of stromal oedema (managed with topical corticosteroid therapy, with complete clinical resolution after few weeks);
- A little epithelial oedema, giving a superficial greyish aspect, was present sometimes soon after the treatment, and reverted in a few days;
- A stabilization of corneal and refractive parameters was observed in all cases, throughout the observation period;
- No endothelial cell loss was detected.
- A statistically significant ($P<0.05$) improvement of some corneal, refractive, and visual acuity parameters was evident after treatments; specifically:

	After 6 months	After 12 months	After 24 months
average UCVA (logMAR)		0.56 vs 0.87	
average BSCVA (logMAR)	0.01 versus 0.103	0.002 vs 0.103	0.01 vs 0.103
average CYL (diopters)		-2.30 vs -3.25	-1.90 vs -3.25

- A significant ($P<0.05$) decrease of the average MEAN K (MEAN K DECREASE) respect to pre-treatment values:
  - At 12 months: 0.58 diopters;
  - At 24 months: 0.59 diopters;

- A significant ($P<0.05$) decrease of the average sphero-equivalent correction (SE DECREASE) respect to pre-treatment values:
  - At 6 months: -0.50 diopters;
  - At 12 months: -0.60 diopters;
  - At 24 months: -0.60 diopters;

Visante® images showed an hyper-reflective stromal area extending from the outer surface to the inner layers; the central depth of this area was always more than 300 microns.

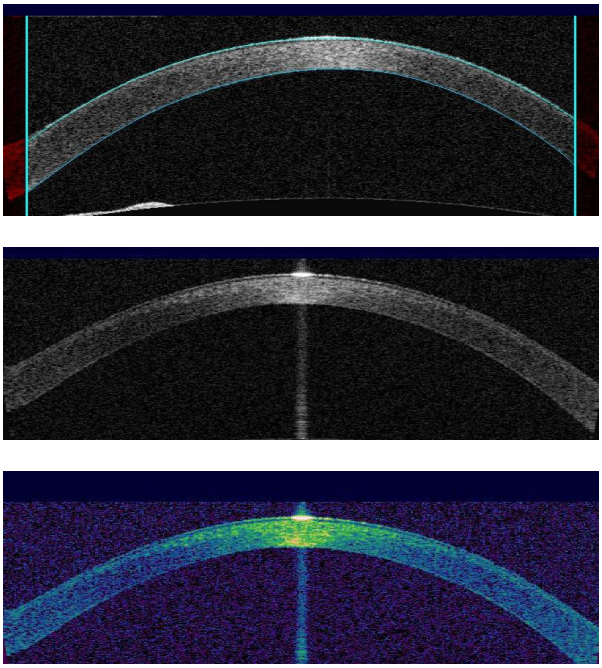

Figure 1 – Details of the stromal area.

Graphic results

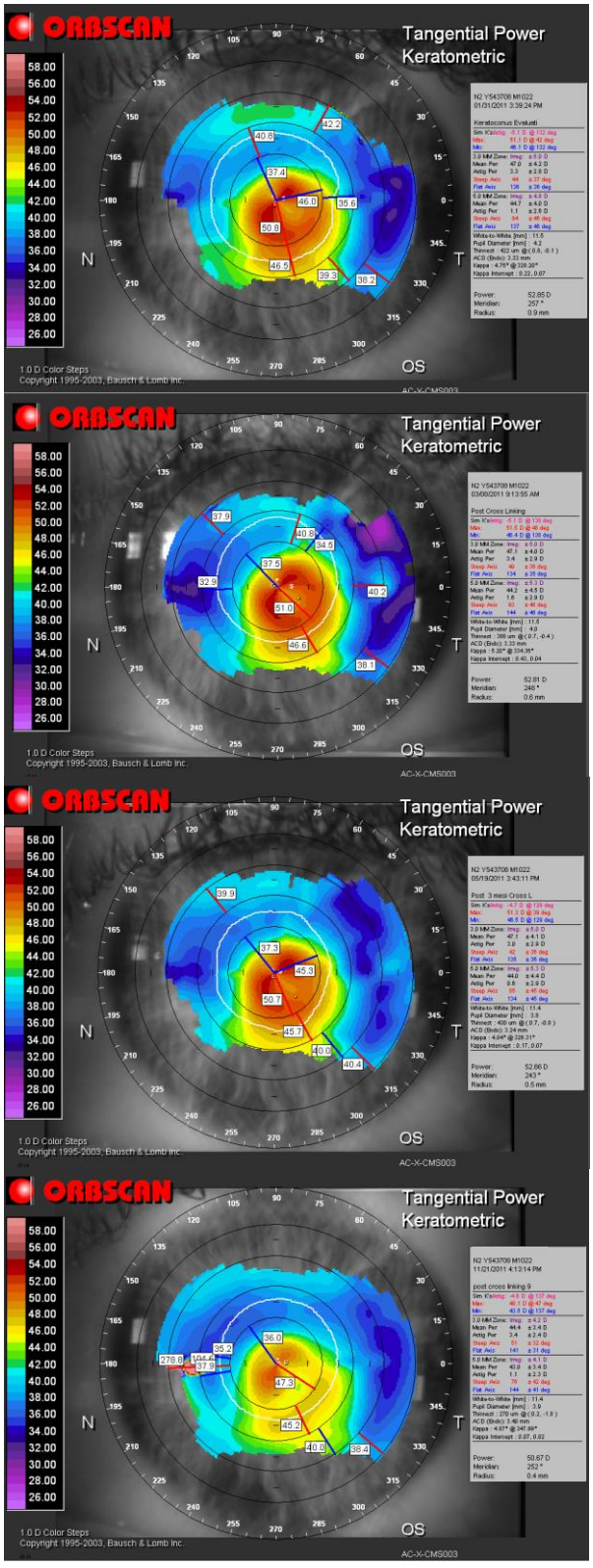

Figure 2 – Example of one case. Sequence: pre, post 3 months, post 6 months, post 12 months.

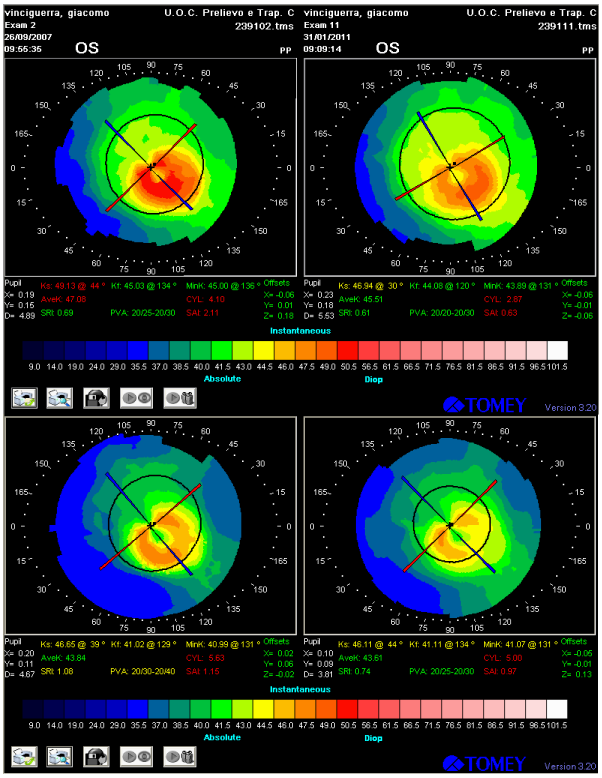

Figure 3 – Pre & post 24 months cases.

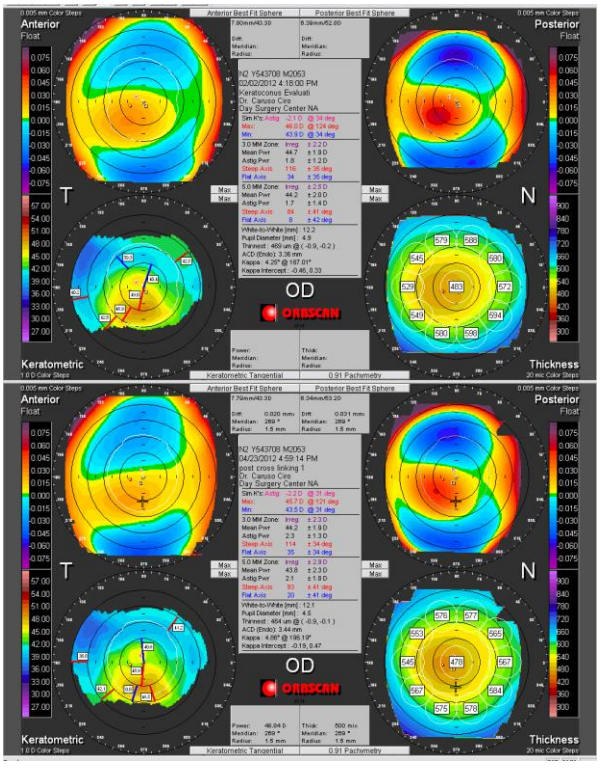

Figure 4 – Pre & post 2 months case.

Discussion

The results of this clinical trial are encouraging:

- The clinical stabilization obtained in all cases, lasting for 24 months, without remarkable side effects.
- Remarkable benefits respect to the traditional TE-CXL protocol:
  a) Reduction of the treatment time respect to traditional protocols (25 minutes versus 60 minutes);
     - More patient compliance;
     - Greater efficiency in doctor and treatment room time;
  b) Less delivery of UV-A radiation:
     - Less risk of damage of the ocular tissues;
  c) More depth of the cross-linking effect in the corneal stroma:
     - Greater lamellar involvement respect to other TE-CXL treatments.

Conclusions

The riboflavin solution for TE CXL is adapted to stop the progression of corneal ectasia, and to improve corneal curvatures and visual acuity. These results were confirmed after two years from treatments, continuing the first year follow-up [2]. Further studies with larger groups of samples are needed, to confirm our results;
Should our results be confirmed in further clinical studies, this solution will be a valid alternative to other solutions used in trans-epithelial cross-linking treatments[3-8].

References

1) Ciro Caruso, Salvatore Troisi, Antonio Del Prete, Luigi Pacente, Gaetano Barbaro, Carmine Ostacolo, Salvatore Del Prete: "*Evaluation of the penetration through human cornea of riboflavin 0.1% in solution with other molecules after trans-epithelial application*" 1st EuCornea Congress, June 17-19 2010, Venice, Italy.

2) Salvatore Del Prete, Ciro Caruso, Robert L. Epstein, Salvatore Troisi, Carmine Ostacolo, Luigi Pacente, Antonio Del Prete, Gaetano Barbaro: "*Thickness and ultra-structural changes in keratoconus-affected human corneas after transepithelial cross-linking*" American Academy of Ophthalmology Annual meeting, October 2010, Chicago (P0073).

3) Ciro Caruso, Carmine Ostacolo, Robert L. Epstein, Salvatore Troisi, Luigi Pacente, Gaetano Barbaro, Antonio Del Prete, Antonia Sacchi: "*A new riboflavin solution for trans-epithelial cross-linking: a study of corneal pharmacokinetic*" American Academy of Ophthalmology Annual meeting, October 2010, Chicago (P0073).

4) Salvatore Del Prete, Ciro Caruso, Robert L. Epstein, Luigi Pacente, Salvatore Troisi, Antonio Del Prete, Gaetano Barbaro: "*Effects of UV-A rays on the corneal epithelial surface after topical application of riboflavin solutions: an electron microscope study*" American Academy of Ophthalmology Annual meeting, October 2010, Chicago (P0073).

5) Carmine Ostacolo, Ciro Caruso, Diana Tronino, Salvatore Troisi, Sonia Laneri, Luigi Pacente, Antonio Del Prete, Antonia Sacchi: "*Increasing of corneal permeation of riboflavin-5'-phosphate through enhancers: A promising approach in corneal trans-epithelial cross-linking treatment*". Inter J Pharm 440 (2013) 148– 153.

6) Gaetano Barbaro, Ciro Caruso, Salvatore Troisi, Antonio Del Prete, Luigi Pacente: "*A Mathematical Model of Corneal UV-A Absorption After Soaking With a Riboflavin Solution During Trans-epithelial Cross-linking*" 1st joint International Congress Refractive Online & SICSSO National Congress, July 7-9, 2011, Grosseto, Italy (Best paper, podium presentation). http://www.irosrc.it/research

7) Ciro Caruso, Salvatore Troisi, Gaetano Barbaro, Carmine Ostacolo, Antonio Del Prete, Luigi Pacente: "*UV-A rays absorption in human corneas before and after trans-epithelial riboflavin application: an experimental study*" 2nd Joint International Congress Refractive Online & SICSSO National Congress, June 28-30, 2012, Rome, Italy (Podium presentation). http://www.irosrc.it/research

8) Ciro Caruso, Robert L. Epstein, Salvatore Troisi, Antonio Del Prete, Luigi Pacente, Gaetano Barbaro: "*Trans-epithelial cross-linking with riboflavin solution: one-year clinical results*" 1st joint International Congress Refractive Online & SICSSO National Congress, July 7-9, 2011, Grosseto, Italy (Podium presentation). http://www.irosrc.it/research